IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

WAGGONER — Atty. Ref.: 4091-2; Confirmation No. 6161

Appl. No. 09/740,486 — TC/A.U. 1639

Filed: December 19, 2000 — Examiner: Ponnaluri

For: CYANINE DYES AS LABELING REAGENTS FOR DETECTION OF BIOLOGICAL AND OTHER MATERIALS BY LUMINESCENCE METHODS

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

EVIDENTIARY DECLARATION UNDER 37 CFR §1.132

I, Alan S. Waggoner, declare that I am the inventor in respect of the above-identified application, and that I am familiar with the Official Action of November 24, 2004. I am also the senior author of the 1981 Biophysical Journal article cited in item 9 of that Official Action.

The dye described in the 1981 abstract does not contain an aryl sulfonate group. It should also be stated that further work performed by me and designed to confirm the degree of labeling of rhodopsin with this dye was not successful. Thus, the absorbance change reported in the 1981 abstract may have been due to dye molecules non-covalently associating with the rhodopsin. As a result of this uncertainty, the work described in the abstract was not further published.

The 1981 abstract makes no mention of the fluorescence properties of the dye because fluorescence was not important to the study, only the absorption of light by the dye. Thus, there is no hint provided in this document that the cyanine dye might be a good fluorescent label.

After 1981, my laboratory became more seriously interested in developing fluorescent labels for detection of proteins, nucleic acids and other biomaterials, especially to quantify the amount of such materials. We tried many ways to develop dyes with adequate extinction coefficients, quantum yields and the ability to absorb and emit light at an easily-detectable wavelength. We came to realize the importance of water solubility of the dye and we incorporated into the dye structure a variety of polar and charged functional groups (but not aryl sulfonation) to solve this problem. The water solubility problem was solved to some degree by these efforts but it became clear that dye-to-dye interactions (dimers and aggregates) of dye molecules on the surface of the labeled material led to quenching of the dye fluorescence. There were no hints or suggestions from dyes or other compounds described in the scientific literature at this time as to how to solve this problem. By 1985 we had tried aryl sulfonation (placing a sulfonate or sulfate group directly on the aryl ring of the dye used in constructing the labeling reagent) and found through protein labeling tests that the aryl sulfonated dyes had acquired both excellent water solubility and were much less likely to form the dye-to-dye aggregates that led to fluorescence quenching. They incidentally had higher quantum yields that benefited fluorescence detection.

Thus, in the 5 or so years between the publication of the 1981 article and the filing of my first U.S. patent application in this series on April 18, 1986, I investigated a wide range of candidate cyanine and related dyes and it took me several years to discover that the aryl sulfonated cyanine dyes have vastly improved properties for fluorescence labeling and detection. Furthermore, they could be synthesized so that they have a molar extinction coefficient of at least 50,000 liters per mole centimeter, an average quantum yields of at least 5 percent and are able to absorb and emits light maximally in the 400 to 900 nm range when bound covalently to a component in an aqueous environment.

When one considers the requirements and properties of the dyes as specified above and the span of years between 1981 publication date of the Biophysical Journal article and the 1986 filing date from which benefit is claimed in the present application, it will be noted it took me several years the to arrive at the present invention and to focus on the aryl sulfonated cyanine dyes having the requisite properties including increased water solubility, stability and reduced dye-to-dye interaction.

I regard myself as being a person having at least ordinary skill in this art. I believe it is an indication of the non-obviousness of the claims now under review that it took me, a highly skilled person in this area, about six years to arrive at the use of the dyes described above, and that on this basis it would have taken one skilled in the art at least as long if not longer to arrive at luminescent dyes having all of the characteristics including an average molar extinction coefficient of at least 50,000 liters per mole centimeter, an average quantum yield of at least 5 percent and a dye that absorbs and emits light maximally in the 400 to 900 nm range when the labeled component is in an aqueous environment.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: March 9, 2005 Alan S. Waggoner